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Award Number: DAMD17-98-1-8582

TITLE: Genistein Programming Against Prostate Cancer

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REPORT DATE: September 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 2000	3. REPORT TYPE AND DATES COVERED Annual (1 Sep 99 - 30 Aug 00)	
4. TITLE AND SUBTITLE Genistein Programming Against Prostate Cancer			5. FUNDING NUMBERS DAMD17-98-1-8582	
6. AUTHOR(S) Coral A. Lamartiniere, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Alabama at Birmingham Birmingham, Alabama 35294-3300 E-MAIL: coral.lamartiniere@ccc.uab.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) We have investigated the potential of genistein, the primary phytoestrogen component of soy, to protect against chemically-induced prostate cancer in rats. Lobund-Wistar rats were exposed to 0, 25 and 250 mg genistein/kg AIN-76A diet, starting at conception and continued until necropsy at 11 months. Male offspring were injected s.c. with Flutamide on days 50-66 to effect chemical castration, with testosterone on days 67-69 to stimulate cell proliferation, with N-methylnitrosourea (NMU) into the dorsolateral prostate to initiate cancer causation, and given testosterone implants, starting at day 77 to promote the cancer. The percent of tumors to the prostate that were classified as invasive adenocarcinomas in rats fed 0, 25 and 250 mg genistein/kg diet were 77.3%, 61.1%, and 44.4%, respectively. Genistein did not alter body, prostate or testes weights or feed consumption. Male rats fed 0, 25 and 250 mg genistein/kg diet had serum genistein concentrations of 9, 60 and 861 pmol/ml, and prostate genistein concentrations of 85, 230 and 775 pmol/g tissue. We conclude that lifetime exposure to "physiological" concentrations of genistein in the diet protected against chemically-induced prostate cancer development in rats without significant toxicity to the offspring.				
14. SUBJECT TERMS Prostate Cancer				15. NUMBER OF PAGES 13
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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INTRODUCTION

Asian men consuming a diet high in soy products have a lower incidence of clinically manifested prostate cancer compared to American and European men (1-3). Soy based diets are high in phytochemicals and quantitative results indicate that genistein is the primary isoflavone constituent of human urine from subjects consuming large amounts of soy products (tofu, soy flour, soy milk, tempeh, etc) (4,5). While the disease of prostate cancer is usually attacked at time of diagnosis, we look at this existing problem from a new perspective. We believe that predisposition to cancer (and its prevention) occurs early in life. We have hypothesized that exposure of male rats to genistein, starting *in utero*, will confer protection against prostate cancer. The aims of this study were: 1) to investigate the potential of life-time exposure to genistein in the diet to reduce susceptibility for prostate cancer, 2) to determine if genistein would regulate the EGFR-signaling pathway as the mechanism of action, and 3) to measure the concentrations of genistein and its metabolites in blood and dorsolateral prostate of male offspring.

BODY

Genistein Exposure. Lobund-Wistar rats were provided 0, 25 and 250 mg genistein/kg AIN-76A diet, starting at conception. Exposure to genistein in the diet, *in utero* until time of carcinogen administration at 70 days postconception, did not cause significant alterations to body weights (Fig. 1). Likewise, perinatal exposure to genistein in the diet did not cause significant alterations to the testes and prostate weights in 70 day old rats (Table 1). The daily feed consumption was measured in young male rats. All animals ate approximately the same amount of feed and gained the same amount of weight (Table 2). From this data we calculated that young male rats fed the low and high genistein containing diets ingested 1.80 mg and 19.25 mg genistein/day/kg BW, respectively.

Prostate Cancer Protocol. From day 50-66 postpartum, male offspring were gavaged with 33 mg Flutamide/kg BW to effect chemical castration. On days 67, 68, and 69, they were injected with 25 mg testosterone/kg BW to stimulate cell proliferation. On day 70, all rats were anesthetized and 42 mg N-methylnitrosourea (MNU)/kg BW was injected into the dorsal prostate for cancer initiation. One week after MNU administration, silastic implants of 25 mg of testosterone were implanted (and replaced every 12 weeks) to stimulate mitosis and promote tumor growth. Animals were necropsied when 48 weeks old or when animals became moribund. Continuation of genistein in the diet to adult rats on the carcinogenesis protocol did not significantly alter body, testes and dorsolateral weights compared to rats fed AIN-76A diet only (Table 3).

Tumor Induction. Except for two animals, one with ganglioneuroma and the other with malignant peripheral nerve sheath tumor, prostatic carcinoma was the sole malignancy identified in these animals. Male offspring exposed from time of conception until 11 months old to genistein in the diet and treated with the carcinogenesis protocol had lower incidence of prostate tumors than those fed the control diet and treated with the same carcinogenesis protocol (Table 4). Rats exposed to 0, 25 and 250 mg genistein/kg diet had tumor incidence of 86.4%, 77.8%, and 63.0%,

respectively. Prostate tumor weights from the 3 groups were not significantly different from one another (5.2 ± 1.8 grams). In animals with small tumors, the tumors were confined to the site of NMU injection, the dorsolateral prostate.

The percent of tumors to the prostate that were classified as invasive adenocarcinomas in rats fed 0, 25 and 250 mg genistein/kg diet were 77.3%, 61.1%, and 44.4%, and the incidence of intraductal carcinomas were 55.5%, 52.9% and 34.5%, respectively (Table 4). Comparison of tumor invasive adenocarcinomas from rats exposed to 250 mg genistein/kg diet to no genistein in the diet was statistically significant to 0.04. In the majority of animals, the tumors presented as Stage I (55%) (confined to the dorsolateral prostate), and in (33%) as stage II and in few (12%) as stage III. Genistein had no effect on the stage of the tumors. Unlike human prostatic carcinomas, which may show all grades, prostatic tumors in these animals presented mostly as poorly differentiated carcinomas. They were composed of small irregular glands and cluster of atypical cells infiltrating a dense stroma. There was extensive acute inflammation, necrosis and calcification. Mucous secretions as well as signet cells were occasionally present. The histology did not vary between animals, or between stages. The lowest score we observed was 6 and the majority between 8 and 10. We found no difference in Gleason's score of tumors in genistein-treated and -untreated animals. Mild to moderate dysplasia was seen less frequently in treated (63%) than untreated (81%) animals.

The prostate tumors did metastasize to the other organs of the reproductive tract, i.e., ventral prostate, seminal vesicles, vas deferens, bulbourethral gland, coagulating gland, epididymis, testes, and sometimes to the penis. There was also metastasis to the lymph nodes, bladder, adrenal gland, diaphragm, kidney, liver, lungs, mesentery, and spleen. There was no evidence of metastasis to the bone or brain. These histopathological findings are consistent with those of Schleicher et al (6).

EGF-receptor expression. Western blot analysis for the EGF-receptor in dorsolateral prostates from rats fed 0, 25 and 250 mg genistein/kg diet did not show any significant differences between the groups.

Bioavailability. Dietary genistein concentrations of 25 and 250 mg genistein/kg diet resulted in serum total genistein concentrations of 60 and 861 pmoles/ml (Table 5). These "frame" the human circulating genistein concentration of Asian men (276 pmol/ml) eating a diet high in soy (7). Enzymatic hydrolysis of the serum with beta-glucuronidase and sulfatase, and HPLC-mass spectrometry analysis (8) revealed that only 15% and 8% of the genistein was free- or aglycone-genistein from blood of rats fed 25 mg and 250 mg genistein/kg diet, respectively, indicating that most of the circulating genistein is conjugated.

In the prostate of rats fed the high genistein containing diet (250 mg/kg diet), genistein concentrations were measured to be 775 pmole/g tissue. This is similar to the corresponding blood levels, 861 pmoles/ml. This shows that there is no genistein transport barrier to the prostate. Another significant finding is that all of the genistein in the prostate was in the free

form (774 pmol/g), none was conjugated. This suggests that all of the genistein is available to the prostate for direct biological action.

Experiments into sex steroid dependent and independent signaling pathways, and down-stream events are necessary. Likewise, investigations into genistein timing of exposure for chemoprevention (prenatal, neonatal, prepubertal and adult) are also warranted.

KEY RESEARCH ACCOMPLISHMENTS

- 1) A rodent model was developed for the study of prostate cancer. Intraprostatic injection of NMU in rats resulted in 77% incidence of invasive adenocarcinomas originating in the dorsolateral prostate.
- 2) Life time exposure to dietary genistein significantly suppressed the development of chemically-induced prostate tumors (invasive adenocarcinomas).
- 3) The concentrations of genistein to exert this chemoprevention resulted in circulating genistein concentrations similar to those found in Asian men eating a diet containing high concentrations of soy. Genistein was bioavailable to the prostate.
- 4) Perinatal exposure to genistein in the diet did not cause significant toxicity to the offspring.

REPORTABLE OUTCOMES

- 1) One abstract in preparation to the American Association for Cancer Research meeting in New Orleans in 2001: Dietary Genistein Suppresses Chemically-induced Prostate Cancer in Lobund-Wistar Rats. Authors: Jun Wang, Isam-Eldin Eltoum and Coral A. Lamartiniere.
- 2) One manuscript in preparation to The Prostate: Dietary genistein suppresses chemically-induced prostate cancer development in Lobund-Wistar rats. Authors: Wang, J., Eltoum, I.-E. and Lamartiniere, C. A.
- 3) Grant proposal in preparation to NCI

CONCLUSIONS

The study of cancer of the prostate is hindered by a lack of adequate animal models. Using intraprostatic injection of the carcinogen, NMU, we have developed a rodent model that resulted in 77% incidence of invasive adenocarcinomas originating in the dorsolateral prostate. Then, we demonstrated that life time exposure to dietary genistein significantly suppressed the development of chemically-induced prostate cancer in rats. Measurements of serum and prostate genistein concentrations showed circulating genistein concentrations similar to those found in Asian men eating a diet containing high concentrations of soy (7), and that genistein was

bioavailable to the prostate. Perinatal exposure to genistein in the diet did not cause significant toxicity to the offspring. This data supports the epidemiological reports that soy (1-3), or genistein, protects against prostate cancer.

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APPENDICES

1 Figure

5 Tables

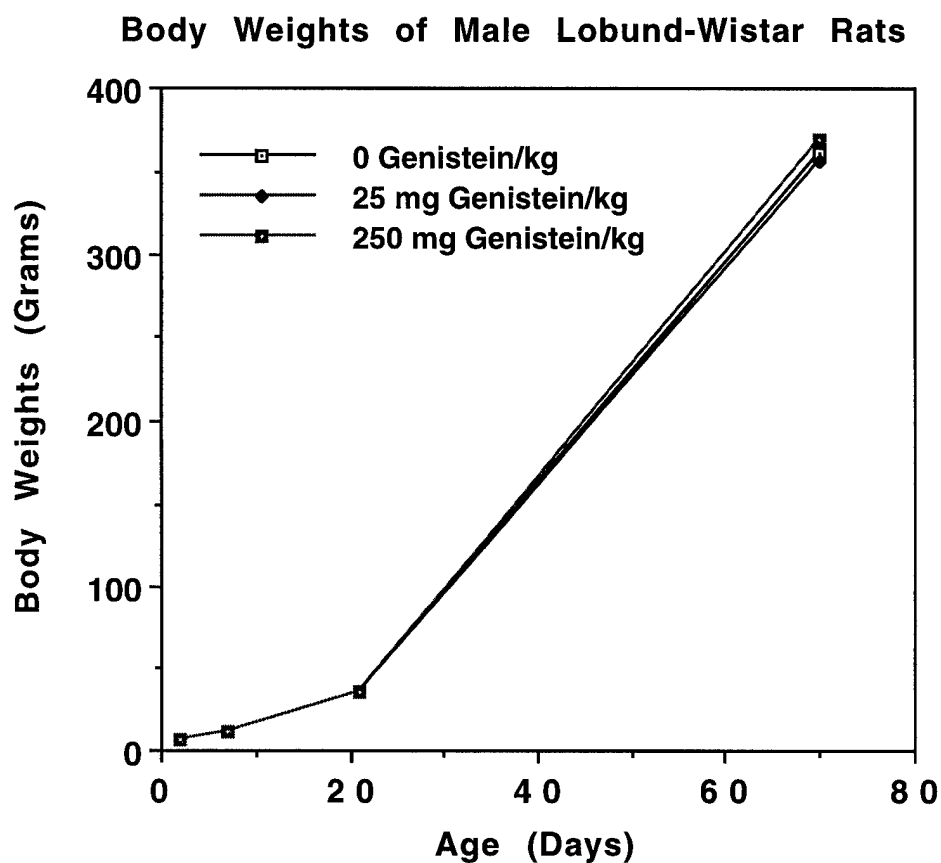


Figure. 1. Body weights of male Lobund-Wistar rats exposed to genistein in the diet from conception until 70 days old. The dams were fed AIN-76A diet supplemented with 0, 25 and 250 mg genistein/kg diet and the offspring were continued to be fed this diet after weaning at day 21 postpartum.

Table 1. Body, Testes and Dorsolateral Prostate Weights in Lobund-Wistar Rats Exposed to Genistein in the Diet from Conception until 70 Days Old*

<u>Diet</u>	Body Weights (G)	<u>Testes</u> (G)	Dorsolateral Prostate (mg)
Zero Genistein in AIN-76A	244 ± 8	2.30 ± 0.04	190 ± 4
25 mg Genistein/kg AIN-76A	256 ± 4	2.35 ± 0.04	188 ± 5
250 mg Genistein/kg AIN-76A	254 ± 3	2.33 ± 0.02	186 ± 5

*Dams were fed AIN-76A diet supplemented with 0, 25 and 250 mg genistein/kg diet and the offspring were continued to be fed this diet after weaning at day 21 through day 70 postpartum.

Table 2. Dietary Genistein Consumption in Lobund-Wistar Male Rats*

<u>Diet</u>	<u>Average BW over Period</u>	<u>Grams of Diet Eaten/Day/Rat</u>	<u>Grams of Diet /Day/kg BW</u>	<u>Grams of Genistein Eaten/Day/kg BW</u>
Zero mg genistein/kg diet	232 ± 6	17.9 ± 0.7	77	0
25 mg genistein/kg diet	235 ± 6	17.0 ± 0.6	72	1.80
250 mg genistein/kg diet	231 ± 6	17.7 ± 0.8	77	19.25

*Dams were fed AIN-76A diet supplemented with 0, 25 and 250 mg genistein/kg diet and the offspring were continued to be fed this diet after weaning at day 21 through day 70 postpartum. Feed consumption was measured from 60-70 days postpartum. Each value represents 8 rats/group.

**Table 3. Body, Testes and Dorsolateral Prostate Weights in
Lobund Wistar Rats Exposed to the Chemoprevention Protocol***

<u>Diet</u>	<u>Body Weights</u> <u>(G)</u>	<u>Testes</u> <u>(G)</u>	<u>Dorsolateral</u> <u>Prostate (mg)</u>
Zero Genistein in AIN-76A	362 ± 8	1.22 ± 0.15	319 ± 17
25 mg Genistein/kg AIN-76A	357 ± 4	1.24 ± 0.18	264 ± 17
250 mg Genistein/kg AIN-76A	369 ± 3	1.59 ± 0.20	242 ± 22

*Rats were exposed to genistein via the diet starting at conception. From day 50-66 postpartum, male offspring were gavaged with 33 mg Flutamide/kg BW. On days 67, 68, and 69, they were injected with 25 mg testosterone/kg BW. On day 70, all rats were injected with 42 mg NMU/kg BW into the dorsolateral prostate. One week after NMU administration, silastic implants of 25 mg of testosterone were started. Animals were killed when 117 days old.

Table 4. Prostate cancer incidence in Lobund-Wistar rats fed genistein in the diet

<u>Treatment</u>	<u>Percent of Rats with Prostate Tumors</u>	<u>Tumor Invasive Adenocarcinomas</u>	<u>Intraductal Carcinomas</u>
0 Genistein in AIN-76A diet	19/22 (86.4%)	17/22 (77.3%)	11/22 (55.0%)
25 mg Genistein/kg AIN-76A Diet	14/18 (77.8%)	11/18 (61.1%)	9/18 (52.9%)
250 mg Genistein/kg AIN-76A Diet	17/27 (63.0%)	12/27 (44.4%) ^a	8/27 (34.5%)

Lobund-Wistar rats were provided 0, 25 and 250 mg genistein/kg AIN-76A diet starting at conception. Male offspring were injected s.c. with 33 Flutamide/kg BW on days 50-66, with 25 mg testosterone/kg on days 67-69, with 42 mg NMU/kg into the dorsolateral prostate on day 70, and given testosterone implants of 25 mg each starting at day 77 (and replaced every 12 weeks). Animals were necropsied when 48 weeks old or when moribund. ^a $P < 0.05$ compared to 0 genistein in AIN-76A diet group (Fisher Exact Test).

**Table 5. Genistein Concentrations in Blood and Prostates of
Lobund-Wistar Rats Exposed to Genistein in the Diet
From Conception until 70 Days Postpartum***

<u>Diet</u>	<u>Genistein Concentration</u>			
	<u>Blood (pmoles/ml)</u>		<u>Prostate (pmoles/g)</u>	
	<u>Free</u>	<u>Total</u>	<u>Free</u>	<u>Total</u>
Zero Genistein in AIN-76A	ND	9 ± 1	ND	85 ± 16
25 mg Genistein/kg AIN-76A	9 ± 1	60 ± 6	ND	230 ± 107
250 mg Genistein/kg AIN-76A	67 ± 7	861 ± 104	774 ± 273	775 ± 246

*Dams were fed AIN-76A diet supplemented with 0, 25 and 250 mg genistein/kg diet and the offspring were continued to be fed this diet after weaning at day 21 through day 70 postpartum. Serum and dorsolateral prostate samples were processed and assayed for genistein concentrations by HPLC-MS. ND: not determined. Each value represents 6 samples/group.